# The Sea Urchin Egg Cortex and Its Transformation After Fertilization

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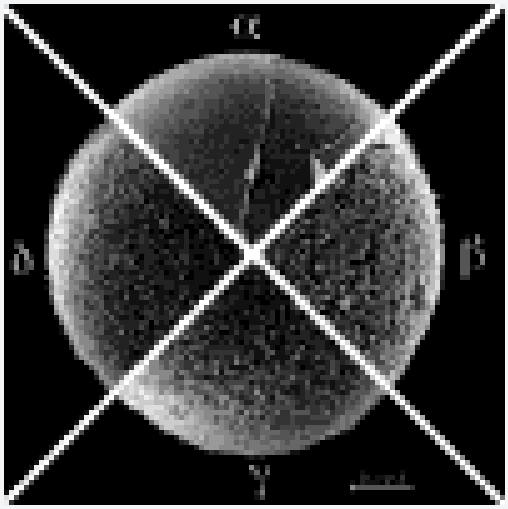
*The cortical transformation creates a permanent block to polyspermy around the sea urchin egg after fertilization.*



***A composite of SEM images of plasma membrane surfaces of eggs at selected stages. a: before fertilization with the fertilization membrane revealed where the vitelline layer is torn away; b: 1 minute after fertilization, when the surface is obscured by globules from the cortical granules; g: 5 minutes after fertilization, following removal of the hyaline layer precursor; and d: 13 minutes after fertilization, when the presence of a few long microvilli give distinctive "fuzzy" appearance. The two early eggs (a and b) were fixed from sea water; later eggs (g and d) were fixed from calcium-free sea water after treatment (Schroeder, 1979).***


## Abstract

The cortical reaction forms a permanent block to polyspermy shortly after fertilization. This process transforms the egg's cortex by releasing enzymes and other proteins into the perivitelline space between the plasma membrane and the vitelline envelope. These enzymes cross-link components in the vitelline envelope and harden it to form a protecting fertilization envelope. This paper describes the cortical reaction and reviews several recent journal articles regarding this important process.


## Introduction

For more than a century researchers have studied the events occurring between fertilization and first cleavage. The model system for this work is primarily the sea urchin. Their prolific production of gametes and subsequent external fertilization makes sea urchins easy to study.

The prevention of polyspermy is one of the most important events occurring after fertilization. Sea urchin eggs accomplish this in two ways. Within 30 seconds after a sperm binds to the egg plasma membrane, a change in the egg's electrical potential blocks entry of other sperm. The second block involves a radical transformation of the egg cortex. This paper describes details of this cortical transformation. A review of some current research in this field follows summaries of fertilization and the cortical reaction.

### Overview of Fertilization

Fertilization is the process whereby sperm and egg unite to form a diploid cell. At fertilization, several important events occur which sends the embryo into the developmental process.

When the sperm contacts the egg, it undergoes an exocytotic event called the acrosome reaction. Hydrolytic enzymes released from the sperm's acrosome enable it to penetrate the egg's jelly coat and reach the plasma membrane (PM). Actin polymerization in the sperm's head causes its elongation toward the egg's PM; this is the acrosomal process. The two PMs unite and the sperm enters the egg.

At sperm-egg fusion, the fast block to polyspermy occurs, followed by the slow block. The fast block, caused by depolarization the egg PM, lasts about 30 seconds. The cortical reaction creates the slow block; a wave of free cytoplasmic calcium initiates it. During the cortical reaction, cortical granules (CG) release their contents into the perivitelline space. These enzymes cause separation of the vitelline envelope; it then rises away from the egg surface. Other CG enzymes then cross-link components of the vitelline envelope (VE) and harden it, thus transforming it into the fertilization envelope (FE).

After the sperm enters the egg, its pronucleus migrates toward the female pronucleus. A diploid embryo results from their union. First cleavage then occurs and the organism begins development.

### Overview of the Cortical Reaction

The cortical reaction, as shown in Figure 1, starts shortly after fertilization when the fast block to polyspermy is still in effect. CGs begin exocytosis at the site of sperm entry and progress around the egg in a coordinated wave. Accompanying this wave is another chain reaction--a wave of intracellular calcium release. This calcium wave initiates the cortical reaction. The CGs release their contents into the perivitelline space when they fuse with the egg's PM. This doubles the egg's surface area.

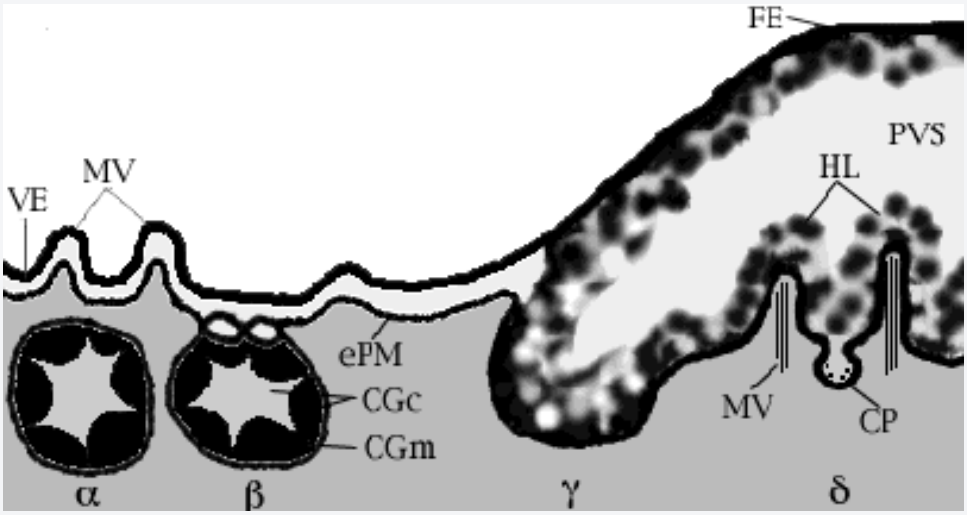



**Figure 1. Schematic representation of the cortical reaction and FE elevation (Giudice, 1986). *alpha*: before fertilization. *beta*: the cortical granule membrane (CGm) fuses with the egg plasma membrane (ePM). *gamma*: the cortical granule contents (CGc) are discharged into the perivitelline space (PVS). *delta*: the fertilization envelope (FE) and the hyaline layer (HL) are formed with the contribution of the CGc and the VE; microvilli (MV) have also elongated and endocytosis via coated pits (CP) is occurring.**

When floppy microvilli (MV) elongate and become rigid, they use some of this extra membrane. These MV elongate when G-actin monomers polymerize and form F-actin (Figure 1, d).

At this time, CG contents are reacting with, and hardening, the VE to form the FE, the slow block to polyspermy. CG secretions first cleave linkages between the VE and the PM. The VE then elevates and other CG constituents form cross-links to harden it. The VE becomes the FE and protects the developing embryo from environmental insults.

Hyalin is another CG component. After CG exocytosis, a hyaline layer forms between the PM and the FE (Figure 1, d). The function of the hyaline layer is not known, but may be involved in morphogenic changes during embryo development.

Finally, receptor-mediated endocytosis via coated pits occurs in the egg's PM. It begins after CG exocytosis and lasts 3-5 minutes. At the end of this time, MV uniformly coat the egg's surface and no CG pits remain. This indicates the function of endocytosis is not only to retrieve extra membrane, but also to selectively take-up portions of the PM components.

The cortical reaction, therefore, is an important and complex event. At the completion of this process, the fertilized egg contains a permanent block to polyspermy and a shield to the outside environment. Not until the embryo releases a hatching enzyme will any substance cross this barrier.

Researchers have appreciated the importance of this reaction for many decades and have extensively studied the cortical transformation. Following is a review of several interesting recent journal articles concerning the cortex and its transformation in sea urchin eggs.

## Matrix Disassembly

Cortical granule matrix disassembly occurs in several distinct stages. After fusion of the CG membrane with the egg PM, a small pore forms. This produces a connection between the cortical contents and the extracellular space. The pore then widens and matrix disassembly completes with fragmentation of the matrix lamellae. Merkle and Chandler (1991) studied these processes by changing both osmolality and divalent cation concentrations in the surrounding media.

Hyperosmolality arrested matrix disassembly by preventing pore widening. During exocytosis, CGs swelled and the pore widened. Since a lack of water stopped this process, Merkle and Chandler suggest water flow is the driving force in this stage of disassembly. For this to occur, a semipermiable membrane is necessary. These researchers speculate the CG contents themselves function as the osmotic barrier. Water availability, however, had no effect after a critical point in disassembly.

During later stages, when CG membranes bind with the PM, the presence of $Ca^{++}$ and $Mg^{++}$ blocks completion of disassembly. Merkle and Chandler determined the matrix lamellae cannot fragment unless these divalent cations leave the CG contents after swelling has occurred. The exocytic pockets can then flatten and release their contents.

## The Two Domains of the Fertilization Envelope

The permanent block to polyspermy occurs when CG contents modify the vitelline layer (VL) after it separates from the egg surface. During this time MV extension is also occurring. These MV casts are distinguishable from the surrounding areas. Based on enzymatic requirements for hardening, Mozingo and Chandler (1991) found two distinct domains within the FE of sea urchin eggs. The first domain being the MV casts and the area between them the second.

To determine these two domains, Mozingo and Chandler used three enzymes important in FE assembly. Linkages between the PM and the VL are first severed by a CG protease. Then, a transglutaminase cross-links VL amine groups. Finally, the FE hardens when ovoperoxidase cross-links dityrosine. By inhibiting each of these enzymes, Mozingo and Chandler could determine FE domains by examining the resulting structures with electron microscopy.

The MV casts use a different set of theses three enzymes during FE assembly than does the surrounding domain. The casts require CG proteases and transglutaminase, but the area between casts needs only ovoperoxidase. Both of these reactions, however, do the same job; they both incorporate paracrystalline material into the FE. Mozingo and Chandler suggest ovoperoxidase-mediated cross-linking of paracrystalline material causes FE hardening.

## Membrane Uptake by Coated Vesicles

During CG exocytosis, large amounts of membrane are added to the egg's surface. MV use some of this extra membrane when they elongate. Fisher and Rebhun (1983) determined endocytosis via coated pits retrieves the remainder of the membrane immediately after MV elongation.

This endocytic uptake remodels the surface membrane by selectively removing membrane components. After MV elongation, the membrane consists of groups of MV separated by smooth areas of CG membrane. Following the 3-5 minute endocytic burst, MV are uniformly distributed across the membrane. Fisher and Rebhun determined that about half of the surface added by CG exocytosis is resorbed by this coated-vesicle uptake.

Fisher and Rebhun also found this membrane uptake is likely $Ca^{++}$-dependent. An intracellular pH rise occurs just after fertilization; this is likely due to the activation of a $Na^{+}$-$H^{+}$ antiport (Epel, 1978; cited in Fisher and Rebhun, 1983). Fisher and Rebhun activated sea urchin eggs in choline sea water that inhibits this antiport-mediated acid secretion. The endocytic burst under these conditions appeared normal. Therefore, endocytosis initiation is alkalinization-independent and likely a $Ca^{++}$-dependent mechanism.

## Endocytosis and Microvillar Elongation

Though endocytosis and MV elongation follows CG exocytosis, they are not necessarily mutually dependent. Could development continue without exocytosis though? Fisher *et. al.* (1985) used a high-pressure technique to answer this question and determine the relationship between these events.

This method employs hydrostatic pressures of 6000-7000psi shortly after fertilization. Application of pressure for 5 minutes blocks CG exocytosis. It blocks all exocytosis except for a small amount around the sperm entry point.

Due to this blockage, three major effects occurred into the first cleavage. First, the normally occurring endocytic burst (Fisher and Rebhun, 1983) and MV elongation were also initially blocked. Second, about 20 minutes after fertilization, endocytosis resumed over the entire egg. Third, about halfway into the first cleavage (30 minutes) MV elongated and endocytic rates increased by several times. The surface after the first division then appeared similar to unpressurized eggs; moreover, they developed into normal larvae.

Thus, CG exocytosis is not required for normal development. CG exocytosis presumably triggers MV elongation and membrane retrieval and normal development is dependent upon them.

## Cytoskeleton Filament Systems in Oocytes

Sea urchin oocytes have an animal-vegetal polarity due to asymmetries in their contents. These gradients may be responsible for pattern formation in early embryos. While their origin is not clear, cortical arrays of microfilaments (MF), microtubules (MT), and intermediate filaments (IF) may play some role in this organization. Using fluorescently-labeled antibodies, Boyle and Ernst (1989) found elaborate fiber networks in sea urchin oocytes.

During egg maturation all three of these cytoskeletal filament systems experience extensive reorganization. Boyle and Ernst used phalloidin binding to show the change in MFs. MFs rearrange from a dense cortical network to a loosely organized network after meiosis.

Their antibody stains show MTs also lose organization. They found MTs both in the cortex and in association with the microtubule organizing center (MTOC) near the germinal vesicle (GV). After meiosis, the number and length of MTs decrease until only MTOC-associated MTs are present. Boyle and Ernst suggest MTs anchor the MTOC and the GV to the PM.

The IF they found in the cortex is a keratin-like filament. It, too, disappears during meiotic maturation. This cortical IF has a single focal center coincident with the MTOC. They suggest, however, that this focal center is not MTOC-dependent.

## Regulation of Respiratory Bursts

Crosslinking of the FE involves production of H2O2 from oxygen during a respiratory burst. This cross-linking occurs when H2O2 reacts with a peroxidase released from CGs during exocytosis. Heinecke and Shapiro (1990) determined protein kinase C activates this respiratory burst after the $Ca^{++}$ rise. FE assembly, however, is independent of this messenger. Figure 2 shows the model developed by Heinecke and Shapiro. It shows the $Ca^{++}$ rise occurs before regulation of the respiratory burst. Initiation of exocytosis, therefore, is not dependent upon protein kinase C.

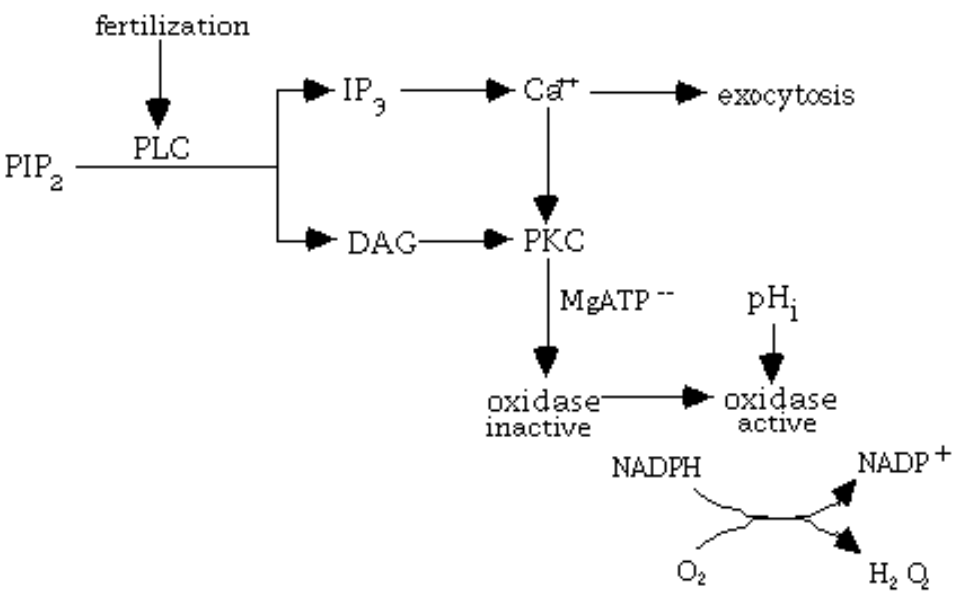



**Figure 2. Model for regulation of the respiratory burst in sea urchin eggs (Heinecke and Shapiro, 1990). Abbreviations: PKC, protein kinase C; IP3, inositol triphosphate; DAG, diacylglycerol; PLC, phospholipase C.**

## Location of Hyalin in the Unfertilized Egg

Though the hyalin layer forms between the PM and the FE during the cortical reaction, it does not necessarily come from CGs. Some researchers have proposed hyalin is instead a membrane protein (McBlaine and Carroll, 1977 and 1980, cited in Hylander and Summers, 1982). Using gold particles coated with anti-hyalin antibodies, Hylander and Summers (1982) determined hyalin does indeed come from CG contents.

They produced hyalin antibodies by immunizing rabbits with purified hyalin protein. The purification procedure involved using a reversible precipitation process. Hyalin precipitates in the presence of 20-50mM $Ca^{++}$ and resolubilizes in $Ca^{++}$-free media. After recovery from the rabbit antisera, they attached the antibodies to gold particles visible under TEM. Hylander and Summers observed the gold particles only in the homogeneous components of the CG in unfertilized sea urchin eggs.

## Cortical Granule Calcium Content and Loss

A simple rise in intracellular free calcium is sufficient to initiate the cortical reaction. The source of this calcium could be the egg's intracellular stores. Using X-ray microanalysis and spectrometric measurements, Gillot *et. al.* (1991) determined the CG's calcium content and its loss at fertilization.

Both of these techniques showed CGs contain a high concentration of calcium (30-95mM) and CGs store 5-11% of the egg's total calcium. Gillot *et. al.* also found a third of the egg's total calcium in the CGs and suggest the rest resides in the endoplasmic reticulum. The decrease in the egg's calcium, furthermore, approximates the amount of calcium in the CGs. However, since egg activation and development can occur without CG exocytosis, CG calcium is unlikely the source of the calcium rise causing the calcium wave at fertilization.

Gillot *et. al.* also suggest an ATP-dependent calcium pump is responsible for accumulation of CG calcium. They further propose a similar pump in the PM causes calcium efflux after exocytosis.

## Origin of Fertilization Envelope Proteins

CG exocytosis secretes proteins into the perivitelline space. The VE then transforms into the hardened FE, thus forming a permanent block to polyspermy. Evidence from previous experiments suggests structural proteins secreted from the CGs bind with the VE to form the FE. Using immunological methods, Villacorta-Moeller and Carroll (1982) found more evidence to support this hypothesis.

Villacorta-Moeller and Carroll found 4 polypeptides in CGs also residing in the FE, but not in the VE. They isolated FEs from fertilized eggs (by using 6M urea!) and used them to produce rabbit antisera. By using antibodies for 8 different FE proteins, they determined the proteins' source.

They found no antibodies bound to the VE or surfaces of unfertilized eggs. Four different antibodies did, however, bind to CG contents. Villacorta-Moeller and Carroll suggest CG contents, specifically a peroxidase, cross-links components of the VE and transforms into the hardened FE.

## Cortical Granule Discharge Sensitivity to Calcium

The wave of CG exocytosis occurs at fertilization starting at the point of sperm entry. Accompanying this wave is a concurrent calcium wave. Researchers speculate this calcium rise is the trigger for the CG discharge; the evidence for this, though, is not conclusive. By using different ionic media, Sasaki (1984) showed the CG discharge exhibits two classes of calcium sensitivity and a 100KDa protein is able to restore a loss in sensitivity.

Sasaki used chaotropic anions (Cl-, Br-, I-, and NO3-) and KCL extracts of the cortex to change the calcium sensitivity of the CG discharge. He suggests the decrease in calcium sensitivity, by a factor of 10, is due the chaotropic effect of these anions. This chaotropic effect changes the properties of water and, hence, the solubility of other substances. By using extracts of cortical contents with KCL, Sasaki could also restore this lost sensitivity. He attributes this restoration to a 100KDa heat and protease labile protein. This protein may be responsible for the propagation of the CG discharge wave.

## The Role of Cortical Spectrin

The cortex of the sea urchin egg undergoes dramatic restructuring after fertilization. These changes continue on through early embryonic development and are thought driven by the cortical cytoskeleton. A major constituent of the cytoskeleton is F-actin; it forms a dense meshwork throughout the cortex. The stability of CGs suggests spectrin may be involved in connecting vesicles to the actin cytoskeleton. By using a plethora of immunological techniques, Fishkind *et. al.* (1990) determined spectrin is important in cortical-cytoskeletal structuring.

Fishkind *et. al.* used immunoblots, immunofluorescence, and immunogold microscopy to determine spectrin's location in 3 classes of cytoplasmic vesicles. They found spectrin on surfaces of CGs, acidic vesicles, and yolk platelets. Furthermore, immunogold assays showed spectrin and actin filament networks in the PM.

These results suggest spectrin acts as a cytoskeleton anchor for CGs, thus giving them stability. Fishkind *et. al.* further suggest spectrin may also form spectrin-ankyrin linkages to ion pumps, transporters, and channels in cortical and plasma membranes.

## Summary

The cortical reaction in sea urchin eggs is more than just the dumping of granule contents into the perivitelline space. This process involves important transformations of the cortex and the surrounding envelopes. Without this transformation, the FE would not form and could not prevent a catastrophic mutli-sperm fertilization. The cortical reaction is a complex process with many implications on later development.

This paper has discussed several events of the cortical reaction in detail. Merkle and Chandler (1991), by changing both osmolality and divalent cation concentrations, determined the CG matrix disassembles in several distinct stages. Mozingo and Chandler (1991) used CG enzymes to find two separate domains in the FE. Fisher and Rebhun (1983) suggest endocytosis via coated vesicles selectively removes PM components. Using hydrostatic pressures of 6000-7000 psi, Fisher *et. al.* (1985) found endocytosis and MV elongation essential for normal development. Boyle and Ernst (1989) demonstrated the existence of an elaborate cytoskeleton consisting of MFs, MTs, and IFs. Heinecke and Shapiro (1990) suggest fertilized eggs produce H2O2 in a respiratory burst, and that protein kinase C activates this oxygen consumption. Ending some controversy about hyalin's origin, Hylander and Summers (1982) found hyalin exists only in the CGs of unfertilized eggs. Gillot *et. al.* (1991) showed CGs store, then release, large amounts of calcium, but this release is unlikely the source of the intracellular calcium wave occurring at fertilization. Using immunological methods, Villacorta-Moeller and Carroll (1982) found proteins in CG contents that later appear in the FE. Sasaki (1984) found that CG sensitivity during discharge decreases in the presence of chaotropic anions, and that a 100KDa protein can restore this loss of sensitivity. Finally, Fishkind *et. al.* (1990) suggest spectrin plays a major role in structuring the egg cortex.

## Appendix

### Abbreviations

| | |
|---|---|
| ATP | adenosine triphosphate |
| CG | cortical granules |
| CP | coated pit |
| F-actin | filamentous actin |
| FE | fertilization envelope |
| G-actin | globular actin |
| GV | germinal vesicle |
| HL | hyaline layer |
| IF | intermediate filaments |
| IP3 | inositol triphosphate |
| MF | microfilaments |
| MT | microtubules |
| MTOC | microtubule organizing center |
| MV | microvilli |
| PM | plasma membrane |
| PVS | perivitelline space |
| TEM | tunneling electron microscopy |
| VE | vitelline envelope |

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